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Genomic organization of a human cystine transporter gene (*SLC3A1*) and identification of novel mutations causing cystinuria

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Genomic organization of a human cystine transporter gene (*SLC3A1*) and identification of novel mutations causing cystinuria. Cystinuria is a common inherited aminoaciduria that leads to recurrent cystine nephrolithiasis. Mutations in a gene encoding a renal amino acid transporter (*SLC3A1*) have been identified in patients with cystinuria establishing one molecular cause for the disease. To facilitate systematic screening of this gene for mutations, we have delineated the complete genomic organization of the *SLC3A1* coding region using polymerase chain reaction strategies. The complete coding region of the gene is contained within a single yeast artificial chromosome clone and consists of 10 exons and 9 introns. Oligonucleotide primers capable of amplifying selected exons have been made and used in mutational analysis of DNA from 24 cystinuria probands. We illustrate the usefulness of this approach by identifying two novel *SLC3A1* mutations. One novel mutation causes replacement of a highly conserved arginine residue (arginine-452) with tryptophan in the cytoplasmic loop between the putative third and fourth membrane spanning segments. A second previously unreported mutation results in replacement of a highly conserved tyrosine (tyrosine-461) residue with histidine in the same region of the protein. In addition, we detected three previously reported *SLC3A1* mutations, R270X, 1500 +1/G toT, and M467T, the latter being present in ~20% of cystinuria chromosomes examined. Our findings provide a foundation for the development of more accessible diagnostic screening assays for detecting *SLC3A1* mutations using patient genomic DNA, and also contribute to the emerging spectrum of cystinuria genotypes.

Cystinuria is an autosomal recessive disorder that leads to recurrent nephrolithiasis. The disease is caused by an inherited defect in the transport of cystine and dibasic amino acids (arginine, lysine, ornithine) across the apical membranes of proximal renal tubular and jejunal epithelial cells and has an incidence of between 1:7000 and 1:12000 [1]. Cystine is poorly soluble in urine and its precipitation results in the formation of stones. Recurrent cystine nephrolithiasis occurs throughout the patient's lifetime and is often associated with significant morbidity.

A candidate gene for cystinuria was identified recently by three groups using expression cloning strategies in *Xenopus* oocytes [2–4]. The ~680 amino acid protein (designated as D2, or rBAT) encoded by this gene exhibits Na^+ independent transport of cystine and dibasic amino acids when expressed in oocytes [2–4], and is localized to the microvilli of the S3 segment of proximal tubule [5]. Human cystine transporter cDNA sequences were reported subsequently [6, 7], and the corresponding gene (designated as *SLC3A1*) has been assigned to human chromosome 2 (2p21) [8, 9]. This chromosomal localization correlated well with that determined by linkage analysis in cystinuria pedigrees [10].

Direct demonstration of *SLC3A1* mutations was first reported in 1994 by Calonge et al [11], thus confirming its role as a cystinuria gene. Several additional mutations have been found in *SLC3A1* which co-segregate with the cystinuria phenotype [12–14]. Functional characterizations of certain missense mutations have revealed reduced transporter activity consistent with the physiologic defect that exists in the disease [11, 12]. In many cases, the detection of *SLC3A1* mutations has relied upon illegitimate transcription of mRNA from this gene in lymphoblastoid cell lines established from peripheral blood leukocytes of cystinuric patients [11–14]. This is a time consuming and labor intensive process which requires an initial delay of several weeks for establishment of the lymphoblastoid lines. Furthermore, this approach is not optimal for delineating mutations affecting critical splice junction sequences. Additional mutations have been found using information regarding the partial genomic organization of the gene [13–15].

To facilitate the ascertainment of *SLC3A1* mutant genotypes, we have defined the complete genomic organization of the *SLC3A1* coding region, and developed exon-specific oligonucleotide primers for use in mutational analysis. We further demonstrate the utility of this approach by identifying five mutant alleles including two novel missense mutations. This information will facilitate the identification of other *SLC3A1* mutations, contribute to our understanding of the molecular basis of cystinuria, and provide the foundation for genotype-phenotype correlations.

Methods

Genomic cloning of SLC3A1

The cDNA sequence coding for human D2 [7] (GenBank accession number M95548) was used to design oligonucleotide primer

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Table 1. Exon-specific polymerase chain reaction oligonucleotide primers for *SLC3A1*

Exon	Forward	Reverse	Size	Temperature
1A	5′-TTATGTCTTCGGAGTGCCTTC	5′-CACCAGCACAGAAGCCACTG	329 bp	52
1B	5′-TACCGCATACCTCGGGAGAT	5′-CTGAAGAACAAAAGAACCATA	256 bp	51
2	5′-AACAAAGTAGGGTTTATTCAT	5′-GAGGGAGGGAGGGAAAGAAA	345 bp	54
3	5′-GACAGGAAAATATACTGATTATT	5′-GGAGAGAAAATAGGTTTGAAT	457 bp	50
4	5′-ATGTGCTGTTTTCTTTGTTT	5′-GAAGTCTGTGTGGGTATCTA	178 bp	52
5	5′-GCCAACCATTTTGCTTCTTC	5′-ACACTCTGATGACATTTTCA	206 bp	52
6	5′-AATGTATGAAATGAGGGTAACC	5′-GCCTGGGAAGAAATGAGAGC	204 bp	54
7	5′-AATCGCTGTCCTCTCCTTCTGTG	5′-TCCTCTCCTTACTCTGCTGTCAT	312 bp	53
8	5′-TCTGTGAAATAGGGTAAATCT	5′-CCTATCAGGTAGAAAACATCT	496 bp	52
9	5′-CTGTTTTCCCTTTCTGGTCTT	5′-GAATAGACAATACTAATCCGTTA	262 bp	52
10A	5′-CCTACTTATTGATGCTTACA	5′-CAGTGTTGATTCTCCAAAAT	344 bp	50
10B	5′-AATCTTTATCGTGGTTCTGA	5′-GCCAGTGTCTTCATCTCTTC	316 bp	49

pairs to amplify *SLC3A1* segments from genomic DNA using the polymerase chain reaction (PCR). Reactions using primer pairs Bf1817/Br1964 (C-terminus; Bf1817: 5′-AATCTTTATCGTGGTTCTGA-3′; Br1964: 5′-TGAGTCCCTCTCCCTTGTCC-3′) and Bf13/Br322 (N-terminus; Bf13: 5′-TTATGTCTTCGGAGTGCCTTC-3′; Br322: 5′-CACCAGCACAGAAGCCACTG-3′) were both successful in amplifying 166 bp (nucleotides 13 to 341) and 329 bp (nucleotides 1817-1982) products, respectively, from human genomic DNA that were co-linear with the human D2 cDNA as determined by direct sequencing. The Bf1817/Br1964 primer set was then used to screen the CEPH megaYAC human genomic library (provided in microtiter well format by Research Genetics, Huntsville, AL, USA). Reactions were performed using 35 cycles of 94°C one minute, 48°C one minute, and 72°C one minute, and a final $MgCl_2$ concentration of 2 mM. Three positive clones were identified. Yeast DNA was isolated from 10 ml liquid cultures by the method of Hoffman and Winston [16] and re-screened for the presence of *SLC3A1* sequences using the Bf1817/Br1964 primer set. Two clones were shown to be false positives. Clone yhCEPH888G9 was positive and was used for delineation of genomic organization.

Definition of SLC3A1 intron-exon boundaries

To screen for intron-exon boundaries, we initially employed vectorette PCR [17]. Yeast DNA isolated from clone yhCEPH888G9 was digested with one of three restriction enzymes (*Alu*I, *Rsa*I, or *Hin*fI), and the resulting restriction fragments were then ligated to specialized double stranded DNA linkers (vectorettes) as described [17]. The resulting vectorette libraries were then used as templates in PCR reactions consisting of an *SLC3A1* coding sequence primer paired with a vectorette specific primer. Single band products obtained in these PCR reactions were directly sequenced after spin column purification (Qiagen, Corp). In the case of multiple products, Southern blot hybridization with a nested [^{32}P] end-labeled oligonucleotide probe was performed. Positively hybridizing products were gel purified, subcloned into the pCRII plasmid vector, verified by colony hybridization, and then sequenced.

To define intron-exon boundaries not fully discerned with vectorette PCR, we utilized either intron-bridging PCR or inverse PCR [18]. For the latter, YAC DNA was digested with one of three restriction enzymes (*Sau*3AI, *Hha*I, or *Taq*I) and the resulting fragments were self-ligated under conditions that favored circularization. PCR primers designed to extend outward in both directions from a known sequence were employed to create products bridging the ligated ends. Products of inverse PCR reactions were processed identically to those of vectorette reactions.

Splice sites were located by identifying consensus splice junction sequences at points of divergence between the cDNA and PCR-generated genomic sequence using computer alignments. For each exon, an overlapping sequence in both directions was obtained. Splice junctions were sequenced in both directions.

Single-strand conformational analysis (SSCA)

Twenty-four blood samples were obtained from patients with well-documented cystinuria as defined by a history of recurrent renal stones and elevated urine cystine level. In most cases, we were unable to assign the allelic type of cystinuria because of the lack of urinary cystine excretion data on related heterozygous carriers. Genomic DNA was isolated by a standard phenol-based method [19]. Exon-specific oligonucleotide primers were designed based upon genomic sequence data, and tested by PCR amplification of human DNA to confirm the expected product size (Table 1).

For SSCA, genomic DNA samples were amplified by PCR (8 min at 94°C, followed by 30 cycles of 94°C for 1 min, annealing temperature for 1.5 min, and 72°C for 1.5 min) using exon-specific primer pairs. Reaction mixtures (final volume 10 μl) contained the following: 200 ng DNA, 0.5 μM each PCR primer, 70 μM each deoxynucleoside triphosphate, 10 mM Tris (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5–2.0 mM $MgCl_2$, 0.25 units of *Taq* DNA polymerase (Boehringer Mannheim), and 0.1 μl of [α-^{32}P]-dCTP (3000 Ci/mmol). Following amplification, PCR products were diluted with 50 μl of 0.1% SDS/10 mM EDTA. A 5 μl sample of each diluted reaction was then mixed with 6 μl of gel loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). Samples were heat denatured at 94°C for five minutes, chilled on ice, and 3 μl loaded onto 0.5X MDE gels (J.T. Baker, Inc.). Gels were electrophoresed 14 hours at 8 watts constant power in Tris-borate-EDTA buffer. After electrophoresis, gels were transferred to blotting paper, dried, and subjected to autoradiography.

Nucleotide sequence determination

Direct sequencing of PCR products was performed using the original amplification primers and the DyeDeoxy terminator cycle

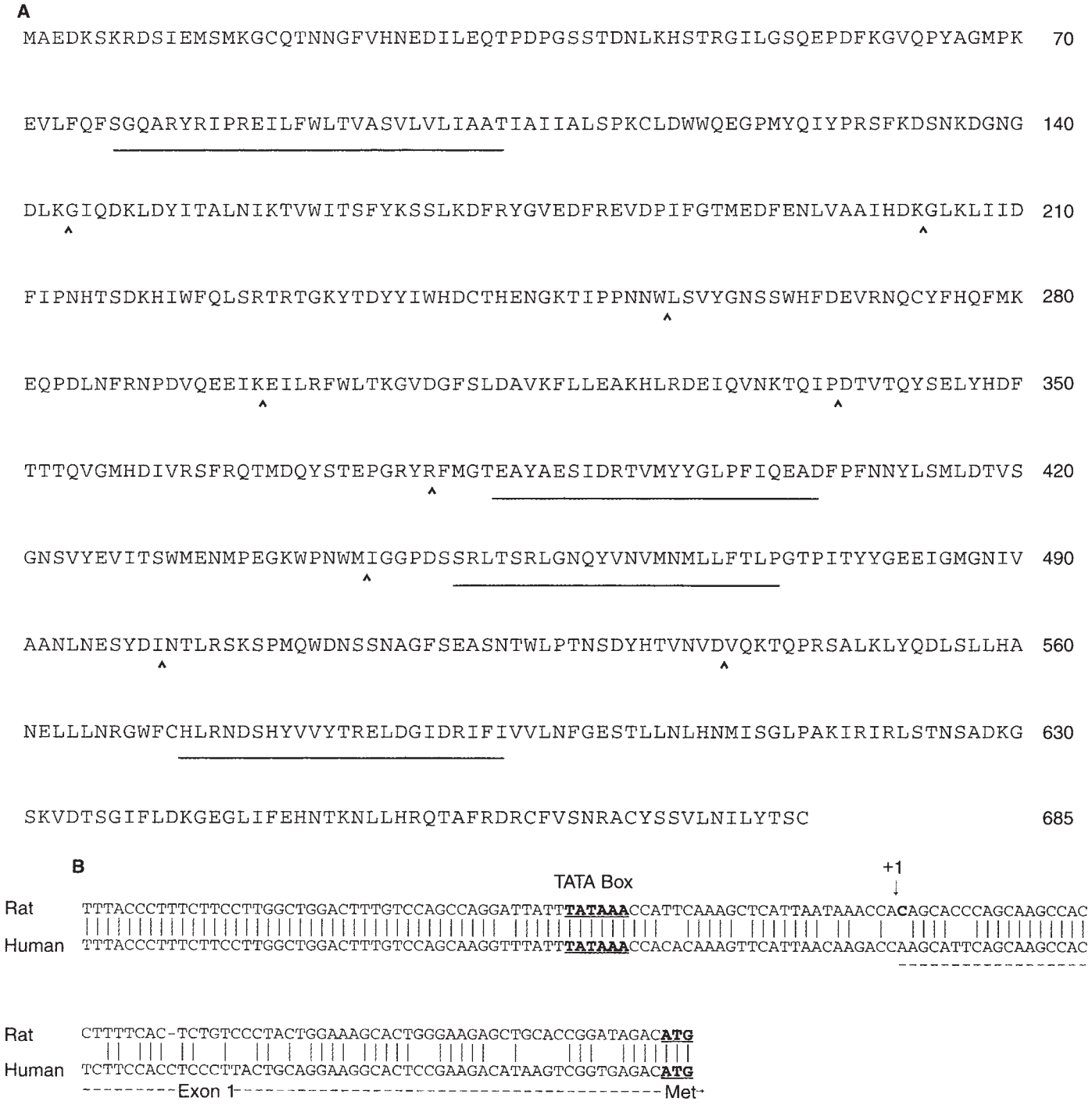

Fig. 1. *Location SLC3A1 introns.* **A.** Amino acid sequence (single letter abbreviation) deduced from the human D2 cDNA [7] shown with intron positions marked by "^" beneath the protein sequence. The locations of the putative membrane spanning segments are shown by horizontal lines below the sequence. **B.** Nucleotide sequence alignment of human and rat *SLC3A1* exon 1 and adjacent 5′ flanking sequence. Transcription start site in the rat sequence is indicated by +1, and a TATA box motif and translation start site (ATG) are indicated by boldface type.

sequencing method (Applied Biosystems, Inc.). Sequencing reactions were electrophoresed and detected on an Applied Biosystems model 377 automated sequencer. For sequencing of abnormal SSCA conformers, individual bands were carefully excised from dried gels, placed into 100 μl of deionized water, and the DNA allowed to elute overnight at room temperature. Ten microliters of eluted DNA were re-amplified using the original primers, and the resulting products were purified by spin column chromatography (Qiagen) before cycle sequencing. Sequence variants were confirmed by sequencing the complementary strand.

Table 2. Catalog of *SLC3A1* splice junctions

Acceptor site[a]	Exon *bp*	Codon	Donor site
	1 (461)	1-143[b]	...GAT CTG AAA G **gt**acatgccc D L K
ttttcttc**ag** GT ATT CAA... G I W	2 (178)	144-203	...CAT GAT AAA **gt**ggggcaag H D K
aacccatt**ag** GGT TTA AAA... G L K	3 (152)	204-255	...AAC AAC TGG **gt**aagatca N N W
tgtttgcc**ag** TTA AGT GTG... L S V	4 (125)	256-297	...GAA ATA AAA **gt**ggagtata E I K
tcatctttag GAA ATT TTA... E I L	5 (119)	298-337	...CAA ATC CCG **gt**aaagtttt Q I P
gttttcaa**ag** GAC ACG GTC... D T V	6 (134)	338-379	...AGA TAC AG **gt**tgaccacg R Y R
actcttat**ag** G TTC ATG GGC... F M G	7 (195)	380-444	...AAC TGG ATG **gt**aagttctc N W M
tgcctttc**ag** ATT GGT GGA... I G G	8 (167)	445-500	...TAT GAT ATT **gt**aagttgaa Y D I
tttgacat**ag** AAT ACC CTT... N T L	9 (116)	501-539	...AAT GTT GAT **gt**aagtatca N V D
aaaaaaat**ag** GTC CAA AAG... V Q K	10 (440)	540-686[c]	

Intron sequences have been deposited in GenBank (Accession numbers U60810-U60819).
[a] Intron sequence in lower case letters, exon sequence with translation beneath in upper case letters
[b] Includes partial sequence of the 5′-untranslated region
[c] Includes partial sequence of the 3′-untranslated region

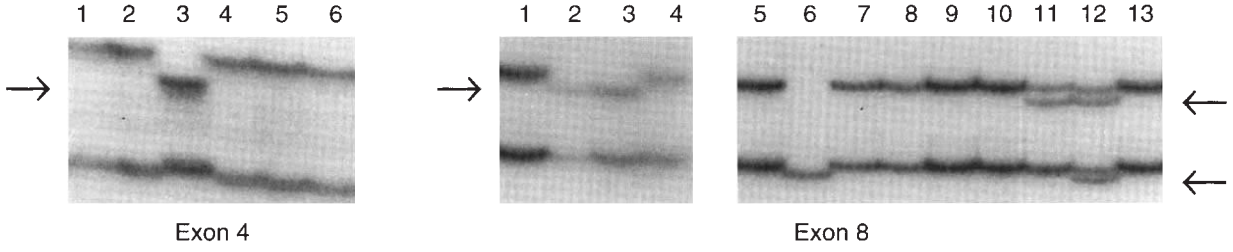

Fig. 2. *Autoradiographs of representative SLC3A1 exon 4 and exon 8 single-strand conformational polymorphisms in several cystinuria probands.* Arrows to the right or left of the panels indicate the electrophoretically abnormal bands.

Allele-specific oligonucleotide hybridization

An oligonucleotide probe (5′-ACAGTTCATGGCTGACT-3′) was synthesized to match the sequence of the R452W allele. Genomic DNAs (200 ng) from affected individuals with the R452W allele and 97 normal controls were amplified using the exon 8 primer pair, and 10 μl of each product were alkali denatured (0.4 M NaOH/25 mM EDTA), then applied to nylon membranes (Hybond-N, Amersham Corp.) using a slot-blot apparatus (Schleicher and Schuell). Membranes were heat and UV fixed then prehybridized for one hour in 5 × SSPE, 5× Denhardt's solution, and 1% SDS. The allele-specific probe was end-labeled using T4 polynucleotide kinase in the presence of [^{32}P]-γ-ATP. Unincorporated nucleotides were removed by G-25 Sephadex spin column chromatography. Hybridizations were done at 48°C for four hours with 0.3 to 0.6 × 10^6 cpm/ml of the end-labeled probe. Filters were washed three times with 1 × SSPE/0.1% SDS for 20 minutes at the hybridization temperature, and subjected to autoradiography.

Results

Genomic cloning of SLC3A1

Without prior knowledge regarding the size of the *SLC3A1* gene, we elected to use a reference human genomic library constructed in yeast artificial chromosomes (CEPH Mega-YAC library) capable of carrying up to 1 megabase inserts. We used a PCR-based screening strategy employing a primer pair designed to amplify a 166 bp product corresponding to part of the carboxyl-terminus of *SLC3A1* from human genomic DNA. This strategy permitted isolation of a single YAC clone (yhCEPH888G9, 940 kb) that contains the gene. This clone contained the complete *SLC3A1* coding region as demonstrated by successfully amplifying sequences from the extreme regions of both the amino- and carboxyl termini.

Definition of intron-exon boundaries

To define splice junctions in *SLC3A1*, we initially employed vectorette PCR using an ordered set of oligonucleotide primers

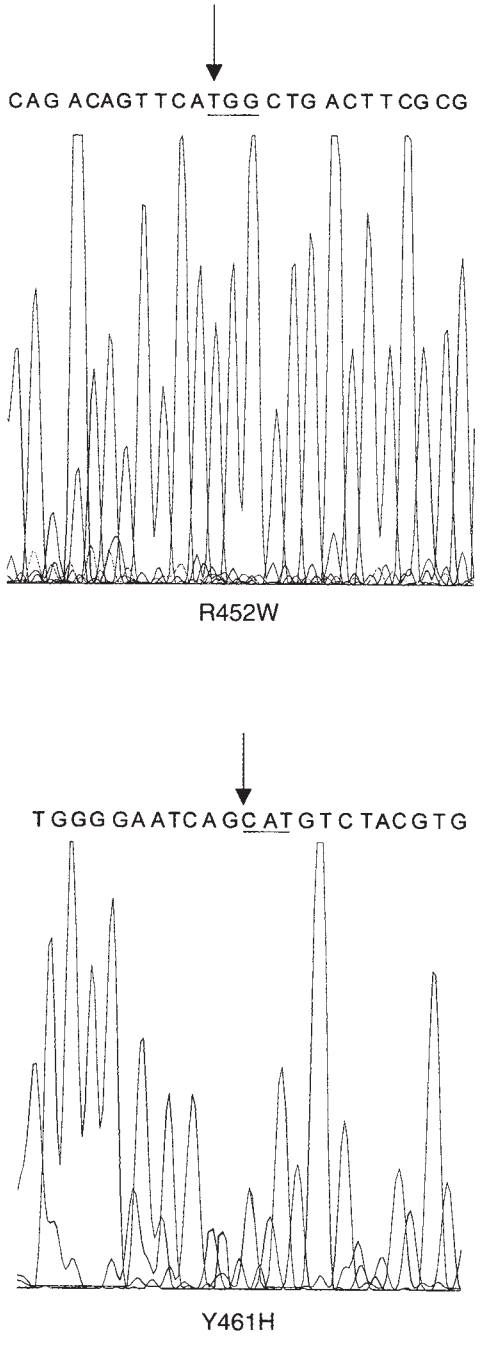

Fig. 3. *Nucleotide sequence chromatographs of two novel SLC3A1 mutations.* Partial nucleotide sequences of R452W (top) and Y461H (bottom) mutant alleles are shown. Vertical arrows indicate the changed nucleotide, and the affected codon is underlined.

designed to anneal at 150 to 200 bp intervals along the coding sequence of the gene on both strands. Vectorette PCR was originally developed to aid in the isolation of end-sequences in YAC DNA [17], but has been used successfully to delineate intron-exon boundaries in complex human genes [20, 21]. Approximately two thirds of the *SLC3A1* coding sequence was isolated using this approach, and nearly all of the splice junctions were identified on at least one intron-exon boundary. To obtain the remaining splice junction sequences, we used either direct intron-spanning PCR or inverse PCR. Use of all three techniques yielded information about the complete intron-exon organization of the *SLC3A1* coding region without the need for high resolution restriction mapping or the subcloning of the YAC clone.

The gene consists of ten exons, ranging in size from 116 to 474 base pairs. The first and last exons are the largest, approximately four times larger than the intervening exons, which are all between 100 and 200 base pairs in size (Fig. 1A). The first exon containing coding sequence was designated as exon 1, and analysis of adjacent 5′ flanking sequence revealed a high degree of nucleotide sequence identity with the proximal promoter region of the rat *SLC3A1* homologue [22] including an experimentally determined transcription start site and TATA box motif (Fig. 1B). In human *SLC3A1,* introns interrupt the reading frame twice (introns 1 and 6). The splice junction sequences are provided in Table 2. All intron-exon boundaries conform closely to the consensus pattern observed in eukaryotic genes [23]; all introns begin with the canonical GT dinucleotide and terminate in an AG dinucleotide. The genomic sequence of *SLC3A1* was co-linear with the human D2 cDNA sequence except a previously reported difference in codon 446 (GG*A* vs. GG*T*), which does not affect the amino acid sequence, and is a likely polymorphism.

Identification of SLC3A1 mutations

We used the genomic sequence information described above to design oligonucleotide primers to facilitate PCR amplification of individual exons from genomic DNA for single-strand conformational analysis (SSCA). Exons 2 to 9 could be amplified using single pairs of intronic primers, but exons 1 and 10 each required amplification of two overlapping regions to restrict the product size to a range (150 to 250 bp) that provides for optimal SSCA sensitivity [24].

Using SSCA, we identified five different mutations in *SLC3A1.* Figure 2 shows an autoradiograph obtained from non-denaturing gels illustrating the abnormal migration of single-strand conformers in our patients. A common abnormal single-strand conformation occurring in exon 8 was observed in nine patients. Direct sequencing of this allele demonstrated it to be a previously reported missense mutation, M467T [11]. Most of our patients with this mutation are compound heterozygotes with a second as yet undefined mutation.

We also observed three other distinct single-strand conformers in exon 8 (Fig. 2, lower bands in lanes 6 and 12). Patient 22 (lane 12) exhibited compound heterozygosity for M467T and a second distinct abnormal conformer, patient 16 (lane 6), exhibited another unique SSCA pattern, and a third SSCA pattern was observed in a single Swedish proband (data not shown). Sequencing of the conformer in patient 22 (lane 12) revealed a C to T transition at nucleotide 1353 of the human D2 cDNA (Fig. 3, top). This nucleotide change results in the substitution of tryptophan for arginine-452 (designated as R452W), a residue conserved at this position in human, rat, and rabbit sequences. This sequence variant was not detected in any other cystinuric probands or in 97 normal control DNA samples examined by allele-specific oligonucleotide hybridization. The non-conservative nature of R452W and its absence in 194 normal chromosomes suggests that it is a mutation rather than a polymorphism. The nucleotide change responsible for R452W occurs in the context of a CpG dinucleotide (*CG*G), which is a known "hot spot" for mutations [25].

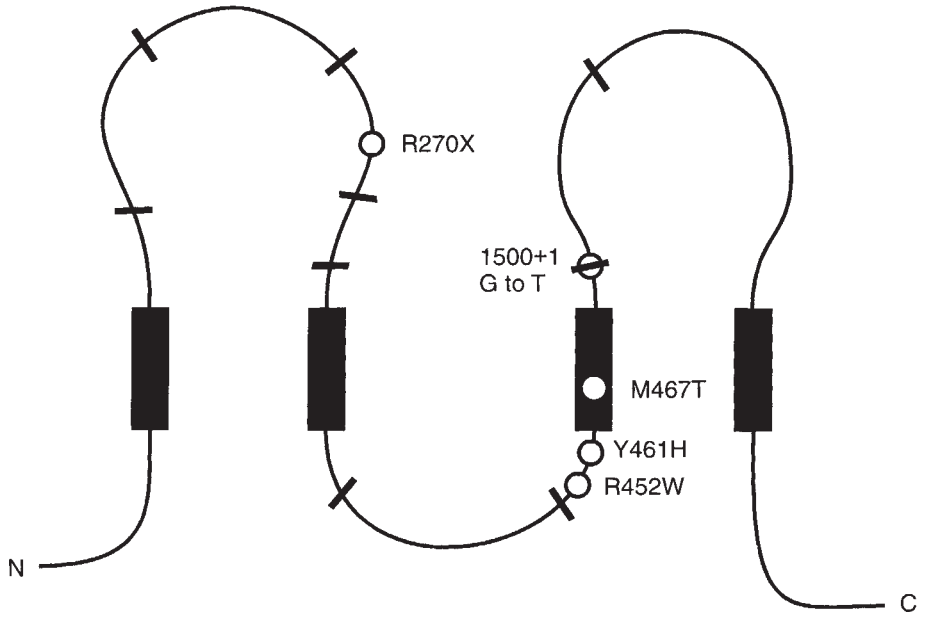

Fig. 4. *Proposed transmembrane topology of SLC3A1 (27) showing locations of five mutant alleles identified in cystinuria probands.* Transmembrane spanning regions are shown by filled rectangles, and the position of introns in the coding sequence are shown by short thick lines intersecting the depicted protein backbone. Mutations are indicated by open circles in their approximate location in the protein.

The abnormal SSCA conformer derived from the Swedish proband revealed a T to C transition at the nucleotide predicting the replacement of tyrosine-461 with histidine (Fig. 3, bottom). Tyrosine is a conserved resdue at this position in human, rat and rabbit sequences. This abnormal conformer was not seen in 94 alleles from patients without the cystinuria phenotype screened by SSCA. The non-conservative nature of the Y461H substitution and its absence in 94 normal chromosomes is consistent with a mutation.

Sequencing of the abnormal exon 8 conformer found in DNA from patient 16 (lane 6) revealed a G to T transversion involving the first nucleotide of intron 8, a mutation previously reported by Horsford et al [15]. This nucleotide change alters the sequence of the splice junction at this location such that the GT dinucleotide normally present at the start of intron 8 is replaced by TT (designated as 1500 +1/G → T). This sequence abnormality is likely to cause aberrant splicing of pre-mRNA transcribed from the gene as inferred from the documented effect of this type of mutation in several other human genes [26].

A fourth distinct mutation was found in exon 4 (Fig. 2). DNA from patient 20 (lane 3, exon 4) appeared homozygous for an abnormal single-strand conformation. Sequencing of the allele revealed a previously reported nonsense mutation at codon 270 (R270X) [14]. Figure 4 illustrates the approximate locations of the mutations and splice junctions reported in this paper using a proposed transmembrane topology model experimentally derived for the *SLC3A1 gene* product [27].

Discussion

Cystinuria is a common inherited disease with an estimated incidence of between 1:7000 and 1:12000 worldwide [1]. Much higher incidence rates are observed in certain ethnic populations. The disorder carries substantial morbidity associated with recurrent nephrolithiasis such as pain, urinary tract obstruction, infection, and loss of renal function. Current pharmacological therapies are directed at solubilizing cystine through disulfide exchange with D-penicillamine or other compounds along with modulating urinary volume and pH. Understanding the molecular basis for this disorder may facilitate the development of additional therapies aimed at replacing the defective gene product.

The first breakthrough in discerning the molecular genetics of cystinuria occurred in 1994 with the identification of mutations in the gene coding for a cystine transporter (*SLC3A1*) [11]. Since then, additional mutations have been defined providing strong evidence that *SLC3A1* is indeed a cystinuria gene [12–14]. However, vast numbers of cystinuria probands exist for whom no molecular genetic data exists. Furthermore, certain allelic subsets of cystinuria as defined by cystine excretion in asymptomatic heterozygous carriers may be caused by mutations at other loci. Recent evidence indicates that type III cystinuria, characterized by intermediate levels of urinary cystine in carriers, is not linked to *SLC3A1*, suggesting the existence of a second cystinuria locus [13]. Clearly more studies are needed to fully document the spectrum of *SLC3A1* mutations and to delineate the basis for the apparent genetic heterogeneity.

We have defined the complete intron-exon organization of the *SLC3A1* coding region to facilitate more rapid screening of cystinuria probands for mutations. Knowledge of the genomic organization of the gene has allowed us to develop a panel of oligonucleotide primers for PCR amplification of each exon directly from patient genomic DNA. This approach obviates the need for the time consuming and labor intensive process of establishing lymphoblastoid lines from patient lymphocytes to examine the *SLC3A1* sequence through use of illegitimate transcripts [11]. This should thus facilitate the systematic detection of *SLC3A1* genotypes in larger populations and may be more amenable to the development of diagnostic screening assays.

We report here two novel *SLC3A1* mutations associated with cystinuria. Both are missense mutations in exon 8 (R452W and Y461H) located in the intracellular loop between the second and third transmembrane spanning segments according to the model proposed by Mosckovitz et al (Fig. 4) [27]. The position of these

mutations are close to that of two other alleles (M467T, M467K) located within the third transmembrane segment. The M467T mutation has been shown to reduce L-cystine transporter activity of recombinant rBAT by ~80% when expressed in *Xenopus* oocytes [11]. The functional effects of M467T and the clustering of mutations in this region of the protein suggest that there are stringent structural requirements within this segment for normal transporter function.

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References

1. MILLINER DS: Cystinuria. *Endocrinol Metabol Clin N Am* 19:889–907, 1990
2. WELLS RG, HEDIGER MA: Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. *Proc Natl Acad Sci USA* 89:5596–5600, 1992
3. BERTRAN J, WERNER A, MOORE ML, STANGE G, MARKOVICH D, BIBER J, TESTAR X, ZORZANO A, PALACIN M, MURER H: Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. *Proc Natl Acad Sci USA* 89:5601–5605, 1992
4. MARKOVICH D, STANGE G, BERTRAN J, PALACIN M, WERNER A, BIBER J, MURER H: Two mRNA transcripts (rBAT-1 and rBAT-2) are involved in system $b^{o,+}$-related amino acid transport. *J Biol Chem* 268:1362–1367, 1993
5. FURRIOLS M, CHILLARON J, MORA C, CASTELLO A, BERTRAN J, CAMPS M, TESTAR X, VILARO S, ZORZANO A, PALACIN M: rBAT, related to L-cysteine transport, is localized to the microvilli of proximal straight tubules, and its expression is regulated in kidney by development. *J Biol Chem* 268:27060–27068, 1993
6. BERTRAN J, WERNER A, CHILLARON J, NUNES V, BIBER J, TESTAR X, ZORZANO A, ESTIVILL X, MURER H, PALACIN M: Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in Xenopus oocytes. *J Biol Chem* 268:14842–14849, 1993
7. LEE WS, WELLS RG, SABBAG RV, MOHANDAS TK, HEDIGER MA: Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic, and neutral amino acid transport. *J Clin Invest* 91:1959–1963, 1993
8. ZHANG X-X, ROZEN R, HEDIGER MA, GOODYER P, EYDOUX P: Assignment of the gene for cystinuria (SLC3A1) to human chromosome 2p21 by fluorescence *in situ* hybridization. *Genomics* 24:413–414, 1994
9. CALONGE MJ, NADAL M, CALVANO S, TESTAR X, ZELANTE L, ZORZANO A, ESTIVILL X, GASPARINI P, PALACIN M, NUNES V: Assignment of the gene responsible for cystinuria (rBAT) and of markers D2S119 and D2S177 to 2p16 by fluorescence in situ hybridization. *Hum Genet* 95:633–636, 1995
10. PRAS E, ARBER N, AKSENTIJEVICH I, KATZ G, SCHAPIRO JM, PROSEN L, GRUBERG L, HAREL D, LIBERMAN U, WEISSENBACH J, PRAS M, KASTNER DL: Localization of a gene causing cystinuria to chromosome 2p. *Nature Genet* 6:415–419, 1994
11. CALONGE MJ, GASPARINI P, CHILLARON J, CHILLON M, GALLUCCI M, ROUSAUD F, ZELANTE L, TESTAR X, DALLAPICCOLA B, DI SILVERIO F, BARCELO P, ESTIVILL X, ZORZANO A, NUNES V, PALACIN M: Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. *Nature Genet* 6:420–425, 1994
12. MIYAMOTO K, KATAI K, TATSUMI S, SONE K, SEGAWA H, YAMAMOTO H, TAKETANI Y, TAKADA K, MORITA K, KANAYAMA H, KAGAWA S, TAKEDA E: Mutations of the basic amino acid transporter gene associated with cystinuria. *Biochem J* 310:951–955, 1995
13. GASPARINI P, CALONGE MJ, BISCEGLIA L, PURROY J, DIANZANI I, NOTARANGELO A, ROUSAUD F, GALLUCCI M, TESTAR X, PONSONE A, ESTIVILL X, ZORZANO A, PALACIN M, NUNES V, ZELANTE L: Molecular genetics of cystinuria: Identification of four new mutations and seven polymorphisms, and evidence for genetic heterogeneity. *Am J Hum Genet* 57:781–788, 1995
14. PRAS E, RABEN N, GOLOMB E, ARBER N, AKSENTIJEVICH I, SCHAPIRO JM, HAREL D, KATZ G, LIBERMAN U, PRAS M, KASTNER DL: Mutations in the *SLC3A1* transporter gene in cystinuria. *Am J Hum Genet* 56:1297–1303, 1995
15. HORSFORD J, SAADI I, RAILSON J, GOODYER PR, ROZEN R: Molecular genetics of cystinuria in French Canadians: Identification of four novel mutations in Type I patients. *Kidney Int* 49:1401–1406, 1996
16. HOFFMAN CS, WINSTON F: A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of Escherichia coli. *Gene* 57:267–272, 1987
17. RILEY J, BUTLER R, OGILVIE D, FINNIEAR R, JENNER D, POWELL S, ANAND R, SMITH JC, MARKHAM AF: A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. *Nucl Acids Res* 18:2887–2890, 1990
18. HARTL DL, OCHMAN H: Inverse polymerase chain reaction, in *Methods in Molecular Biology*, edited by HARWOOD AJ, Totowa, Humana Press, Inc., 1994, p 187
19. SAMBROOK J, FRITSCH E, MANIATIS T: *Molecular Cloning: A Laboratory Manual* (2nd ed). New York, Cold Spring Harbor Press, 1989
20. ROBERTS RG, COFFEY AJ, BOBROW M, BENTLEY DR: Determination of the exon structure of the distal portion of the dystrophin gene by vectorette PCR. *Genomics* 13:942–950, 1992
21. TÜMER Z, VURAL B, TONNESEN T, CHELLY J, MONACO AP, HORN N: Characterization of the exon structure of the Menkes disease gene using vectorette PCR. *Genomics* 26:437–442, 1995
22. YAN N, MOSCKOVITZ R, GERBER LD, MATHEW S, MURTY VVVS, TATE SS, UDENFRIEND S: Characterization of the promoter region of the gene for the rat neutral and basic amino acid transporter and chromosomal localization of the human gene. *Proc Natl Acad Sci USA* 91:7548–7552, 1994
23. MOUNT SM: A catalogue of splice junction sequences. *Nucl Acids Res* 10:459–472, 1982
24. SHEFFIELD VC, BECK JS, KWITEK AE, SANDSTROM DW, STONE EM: The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* 16:325–332, 1993
25. BARKER D, SCHAFER M, WHITE R: Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. *Cell* 36:131–138, 1984
26. KRAWCZAK M, REISS J, COOPER DN: The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: Causes and consequences. *Hum Genet* 90:41–54, 1992
27. MOSCKOVITZ R, UDENFRIEND S, FELIX A, HEIMER E, TATE SS: Membrane topology of the rat kidney neutral and basic amino acid transporter. *FASEB J* 8:1069–1074, 1994